

# NATIONAL BUREAU OF STANDARDS REPORT

9846

Technical Summary Report Number 1

For the Period  
May 1, 1967 to April 30, 1968

Contract NAONR 14-67  
ONR Contract Authority Identification Number NR 036-072

Subject

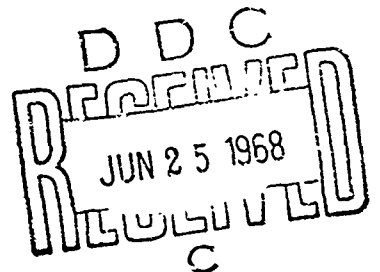
**MICROBIAL CORROSION**

Submitted to the Office of Naval Research

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U.S. DEPARTMENT OF COMMERCE  
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MICROBIAL CORROSION

Warren P. Iverson

National Bureau of Standards  
Washington, D C.

30 April 1968

# NATIONAL BUREAU OF STANDARDS REPORT

## NBS PROJECT

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U.S. DEPARTMENT OF COMMERCE

NATIONAL BUREAU OF STANDARDS

### ABSTRACT

Studies of the effect of microorganisms, particularly sulfate reducers (Desulfovibrio), on marine corrosion were initiated. The inability of sulfate reducers to grow on the agar surface of media appears to be due to the presence of the ferrous salt used as an indicator for hydrogen sulfide. Evidence indicates that phosphate may be an electron acceptor in the cathodic depolarization of steel. Iron phosphide ( $\text{Fe}_2\text{P}$ ) and vivianite ( $\text{Fe}_3(\text{PO}_4)_2 \cdot 8\text{H}_2\text{O}$ ) were produced by Desulfovibrio growing in contact with mild steel. The organism reduces phosphate in the presence of hydrogen to form a volatile phosphorous containing compound which is not phosphine. Light increases the corrosion rate of steel as indicated by polarization measurements. The cathodic protection currents required to maintain a potential of 0.8 volts on a steel specimen in indirect sunlight was found to be 1.5 times that required in the dark.

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## INTRODUCTION

Corrosion in the marine environment continues to be a problem of major importance. The marine environment, because of the high concentration of dissolved salts is a most favorable environment for corrosive action. Obviously the great abundance of flora and fauna in the ocean is a contributing factor to much of this corrosion, particularly the marine microorganisms, because of their wide variety of chemical activities. Just how important the contributions of living forms is to marine corrosion remains to be determined.

There is quite good evidence, however, that bacteria can cause corrosion by bringing about local changes in oxygen or ion concentration or by producing corrosive substances such as acids as a result of their metabolic activities. Although most of this evidence has been obtained using non-marine forms, there is no doubt that marine forms would initiate some reactions since they have been shown to carry out identical chemical reactions.

Certain bacteria, particularly the anaerobic sulfate reducing bacteria (Desulfovibrio, sp.) have been associated with corrosion, probably more than any other group, for a number of years. They have been postulated to cause corrosion, particularly of iron and steel by a cathodic depolarization process in which they remove hydrogen from the metal surface and use the electrons to reduce sulfate to form



hydrogen sulfide. As a result of the hydrogen or electron removal, iron goes into solution (1). It has been demonstrated that this mechanism does take place if the dye, benzyl viologen, is substituted for sulfate as an electron acceptor (2).

One of the objectives of this contract was to study this reaction in greater detail since some previous studies had indicated that an electron acceptor other than benzyl viologen might be involved. As marine sulfate reducers (Desulfovibrio sp.) have been associated with marine corrosion (3-7), a second objective was to obtain them in pure culture and determine if they would cause corrosion by the same mechanism as the non-marine strains.

A third objective was to compare the corrosion process in sea water with and without microorganisms.

#### EXPERIMENTAL WORK

##### 1. Growth and Isolation Studies

It is highly desirable for studies in microbiology or microbial corrosion that pure cultures be used and maintained. This is much more easily accomplished if the cultures can be grown on the surface of agar media rather than in liquid media.

Cultures of the sulfate reducing organism, Desulfovibrio desulfuricans, have been very difficult to grow on an agar surface and are usually grown in liquid media. As a result many, if not all the cultures available,

are or have become contaminated. Most of these liquid media contain sodium lactate as a source of hydrogen or electrons, one or more salts containing sulfate as an electron acceptor, a source of nitrogen such as ammonium chloride, one or more agents to lower the redox potential such as ascorbic acid or thioglycollate, a phosphate buffer, and an iron salt, usually ferrous ammonium sulfate, to act as an indicator of hydrogen sulfide (blackening being due to FeS production). Yeast extract is usually added also, since it affords better growth. Sodium chloride is usually added for cultivation of marine strains. The ferrous indicator salt and the redox lowering agents are usually sterilized separately by filtering and added to the rest of the autoclaved ingredients before inoculation.

Recently, two commercially available media (trypticase soy agar and trypticase soy broth--to which agar is added) were reported to be satisfactory for the surface growth of the organism (Appendix). A different medium (for distinguishing the sulfate reducers from the other microorganisms) was developed using the trypticase soy agar as a base and adding lactate, sulfate and an indicator salt (Appendix). Investigations carried out in this study appear to indicate that the failure of the surface growth on agar using media devised for the growth of the organism (liquid media) is due to the inhibition of growth by the ferrous indicator ( $H_2S$  formation) salt.

(a.) Inhibitory Effect of Ferrous Salt on Agar Growth of Desulfovibrio

In some previous unpublished studies, it was found that only yeast extract plus a hydrogen or electron donor and an electron acceptor would provide excellent growth on the surface of agar. Luxuriant growth of Desulfovibrio was found on agar plates (2%) of yeast extract plus magnesium sulfate (0.2%) in a hydrogen atmosphere. A distinct green color was noted in the agar, probably due to the green pigment produced by Desulfovibrio strains, namely desulfovibrin. If the indicator salt, ferrous ammonium sulfate (sterilized by Seitz filtration) was added, the typical poor growth as described above was noted.

Further evidence of the presence of the inhibitory action of the ferrous salt was obtained. In a private communication from Dr. Postgate, University of Sussex, England, he stated that trypticase soy agar (Baltimore Biological Laboratories) (Appendix), as well as the corresponding Difco product and a medium reported by Miss Pankhurst (London Research Station, Gas Council) at the International Microbiology Congress in Moscow had shown greater promise than any previous techniques using plates. The formulation of the medium obtained from Miss Pankhurst is essentially Baar's medium with a little yeast extract (0.1%) and thioglycollate (0.01%) set with agar (Appendix).

It appears from the formulation that the ferrous ions from ferrous

ammonium sulfate would have been oxidized to ferric ions after steaming for three successive days. Since there were no ferrous ions present, no inhibition would be expected.

A total of 12 petri plates of Miss Pankhurst's medium (2% agar) were prepared. Six of the plates contained ferrous ammonium sulfate (sterilized by Seitz filtration and immediately added to the medium before solidification). The ferrous salt was not added to the remaining six plates. The six plates with the indicator salt were streaked with 3 strains\* of Desulfovibrio (2 plates per strain) and placed in a Brewer jar containing a dish of saturated sodium carbonate and pyrogalllic acid to remove traces of oxygen. The jar was evacuated and replaced with hydrogen three times and incubated at  $28^{\circ} \pm 1^{\circ}\text{C}$ . The same procedure was carried out with the six plates minus the indicator salt.

After incubation for 19 days the plates were removed and examined for growth. Good growth (over the entire area which was streaked) was present on all the plates where the indicator salt was absent. All of the six plates containing ferrous ammonium sulfate with the exception of one were devoid of growth. The agar plate where growth was present had smaller volume of space between the cover and the surface of the agar due to the smaller depth of this particular plastic dish. Possibly enough of the gas,  $\text{S}_2\text{O}$ , produced by the original inoculum, which has a

\*The American Petroleum Institute Strain (Mid-Continent A); a strain isolated from a contaminated jet fuel water bottom; and a strain isolated from a corrosion pit in an aluminum tank. Pure cultures of these strains are maintained on trypticase soy broth plus agar plates under a hydrogen atmosphere at  $28^{\circ}\text{C}$ .

stimulatory effect upon the growth of Desulfovibrio on media containing the ferrous salt accumulated sufficiently in this smaller volume to initiate growth (8).

It appears from these observations that the only essential ingredients for a medium which would provide good surface growth might be: (1) an electron donor, (2) yeast extract and (3) an electron acceptor. Sodium chloride may be necessary for marine strains. Since the ferrous salt appears to inhibit growth a differential medium might be devised which contains the three essential ingredients plus a ferric salt as an indicator of hydrogen sulfide production.

(b.) Isolation and Growth of Desulfovibrio from Marine Fouling

One of the objectives in this investigation was to isolate and study the corrosion mechanism or mechanisms of some marine strains of Desulfovibrio. These organisms have previously been mentioned as being associated with marine corrosion.

Ten 1 ml samples of supernatant fluid from the fouling detritus scraped from five pilings off the Virginia Beach Coast were inoculated into 5 ml vials of API medium (Appendix) within a few hours of sampling. After 3 days incubation at ambient temperature, blackening of the medium was found to occur in all the vials. Bottles of Baar's medium (Appendix) which contained sodium chloride (2.5%) were also inoculated with the samples. Blackening also took place in these bottles within a few days.

As the trypticase soy agar plus salts medium was found to be quite successful for growth of non-marine forms of Desulfovibrio (Appendix), it was used for isolation. Agar (2%) plates of this medium containing 3.0% sodium chloride were streaked from the API medium and incubated under a hydrogen atmosphere. Black areas developed on the plates within one week's incubation. Microscopic examination of growth typical of Desulfovibrio revealed an abundance of spiral forms characteristic of the organism. Repeated efforts involving 9 or 10 transfers (with the possibility of adaptation in consideration) to isolate colonies of Desulfovibrio on this medium using the streak dilution technique were not too successful. Although growth of Desulfovibrio occurred on each transfer, no good separation could be achieved. At present, work is in progress to develop a better isolation medium. The effect of salt concentration will be investigated as well as the use of sea water instead of distilled water for preparation of the medium. Various media used for the growth of Desulfovibrio in liquid cultures containing ferric salts as an indicator of  $H_2S$  will also be employed.

When the marine strains are isolated in pure culture it is planned to investigate their influence on the rate of corrosion of ferrous metals in sea water and their mechanism or mechanisms of corrosion.

## 2. Mechanism of Cathodic Depolarization

As indicated in the introduction, Desulfovibrio has been postulated

to cause corrosion by a process of cathodic depolarization (removal of hydrogen or electrons at the cathode by the enzyme hydrogenase), if a suitable electron acceptor were present. This acceptor was postulated to be sulfate. Benzyl viologen has been substituted for sulfate and found to be a satisfactory electron acceptor in the presence of iron. It seemed of interest to determine whether this compound could replace sulfate as an electron acceptor for growth. The utilization of other electron acceptors (for growth and for cathodic depolarization) was also investigated.

(a) Growth on Yeast Extract Containing Benzyl Viologen and Phosphate as Electron Acceptors

It was previously stated that yeast extract provided an excellent growth medium for Desulfovibrio if an electron donor (such as hydrogen) and an acceptor such as sulfate were present. Little, if any, growth could be found on yeast extract (2%) agar (2%) plates if one or both were absent for incubation periods up to two weeks. Such a medium provides an excellent screening medium for electron donors and acceptors. It was of interest to determine whether yeast extract without any added electron acceptors would support growth of Desulfovibrio if the incubation time was greatly prolonged. It was also of interest to determine whether benzyl viologen was a hydrogen acceptor would promote growth in the presence of hydrogen. Cells of Desulfovibrio have been previously demonstrated to reduce benzyl viologen, but benzyl

viologen (50 mg/ml) has also been reported to inhibit growth of the organisms (9). Two brands of commercially available yeast extract were compared for their inability to support growth in the presence of electron donors and acceptors.

Three plates of Difco yeast extract (2%) plus agar (2%) and three plates of BBL yeast extract (2%) plus agar (2%) were prepared. Three plates of Difco yeast extract (2%) agar (2%) containing benzyl viologen (100 mg/ml) were also prepared. The pH of the agar was adjusted to  $7.0 \pm 1$  in all cases before autoclaving. After streaking all plates with the API strain, the plates were placed in dessicator jars. The three plates containing the benzyl viologen were placed in a separate jar. Petri dish bottoms containing alkaline pyrogallol were placed on top of the stacks of petri dishes in the jars. The jars were evacuated and replaced three times with hydrogen.

After 5 months incubation at 27-28°C, no growth of Desulfovibrio was observed on the plates of BBL yeast extract agar. Good growth, however, was observed on the three plates of Difco yeast extract agar. Microscopic examination of this growth showed many cells to be motile. Very poor growth was found on the three plates of yeast extract agar plus benzyl viologen. Microscopic observation revealed few, if any, intact cells. The benzyl viologen was reduced (violet areas), however, under nearly all of the agar area streaked with the cells.



Benzyl viologen does not appear, therefore, to be a satisfactory electron acceptor for growth of the organism, at least in the concentration used. Preliminary observations would indicate that it might be based upon the considerable reduction of the dye even by very small numbers of cells. It appears that the Difco yeast extract does contain both electron donors and acceptors which are very slowly utilized whereas the BBL product does not. Further experiments with the BBL product are planned since it would appear to be an even better screening agent than the Difco yeast extract.

As it was reported that iron phosphide is produced by Desulfovibrio (vide infra, 10), it appeared from these observations that phosphate might be an electron acceptor. This was verified by observing good growth on three plates of Difco yeast extract (2%) agar (2%) with  $K_2HPO_4$  (0.5 gm/100 ml agar) and the absence of growth on three plates of the same medium without the phosphate, after one week observation under a hydrogen atmosphere at 28°C. (The pH of the yeast extract agar without the phosphate was brought to  $7.0 \pm 1$  by the addition of KOH. The agar containing  $K_2HPO_4$  (pH  $7.0 \pm .1$ ) needed no pH adjustment.) The growth was not nearly as luxuriant as on the yeast extract agar with sulfate as the electron acceptor. Microscopic examination of the growth on the phosphate agar revealed long loosely coiled spirals to predominate instead of the short curved rods, normally found.

(b) Formation of Iron Phosphide by Desulfovibrio

The black materials produced (at Fort Detrick) by Desulfovibrio growing in yeast extract (2%) containing 1010 steel was non-magnetic and no X-ray diffraction pattern was produced from it. Upon heating to 2,250°F for 15 minutes in a vacuum and allowing to cool to room temperature for 17 hours, a diffraction pattern for  $\text{Fe}_2\text{P}$  was obtained. The material had also become magnetic. Comparison of this material with a commercially available sample (Rocky Mountain Research, Inc.) by Mössbauer spectroscopy revealed the materials to be similar.

Comparison of the colloidal non-magnetic material before heating with the commercial preparation by Mössbauer spectroscopy seemed essential. Six 50 ml centrifuge tubes containing 40 ml of 1% Difco yeast extract broth (pH  $7.0 \pm 0.1$ ) and coupons of 1010 steel were inoculated with Desulfovibrio (API strain). After 11 days incubation under a hydrogen atmosphere, the liquid cultures (black) were combined in a centrifuge bottle with a rubber cap. The atmosphere above the culture was replaced with hydrogen. Centrifugation was unsuccessful until the liquid culture was rapidly frozen using dry ice, and thawed. After washing twice in boiled distilled water (to remove most of the oxygen) and drying in a vacuum, 11.6 mg of black powder was obtained. This sample was compared with the Mössbauer spectrum of the heated

sample of both samples appeared identical. It was of interest that microscopic examination of the culture before centrifugation revealed extremely long spiral forms of Desulfovibrio to be present. Some forms extended across the entire field.

Of interest also was the finding of black crystals on the 1010 steel specimens in (2%) yeast extract broth inoculated with the API strain when allowed to incubate at 28°C in a hydrogen atmosphere for a long period of time (1 month). They appeared to predominate on areas not covered with a dark film (presumably iron phosphide). No X-ray diffraction pattern could be obtained from the film (Figures 1 and 2), however.

X-ray diffraction analysis of the crystals revealed them to be vivianite ( $\text{Fe}_3(\text{PO}_4)_2 \cdot 8\text{H}_2\text{O}$ ). No pits were found under the crystals.

At present no definite explanation can be given for their formation although it probably is related to the process of iron phosphide formation or degradation. No similar crystals were noted on steel in uninoculated yeast extract broth inoculated for longer periods of time.

#### (c) Formation of Volatile Phosphorous Compound

The evidence obtained (namely, growth on yeast extract agar containing phosphate and the production of iron phosphide) indicated that the organisms were probably reducing phosphate. Some early investigations had already reported the reduction of phosphate to

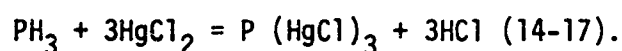
phosphine by some microorganisms (11, 12).

Several attempts to determine the presence of phosphine ( $\text{PH}_3$ ) by infrared spectroscopy were negative although on one occasion a band suggestive of P - H bonds appeared. There was some doubt as to the reliability of the instrument at this particular time, however. In these investigations the hydrogen atmosphere in a Brewer jar containing 9 inoculated (API strain) plates of Difco yeast extract (2%) agar (2%) plus a potassium phosphate (0.5 gm/200 ml agar) was flushed with helium (4-5 hours) through a methanol dry ice trap to remove the moisture and a liquid nitrogen trap to collect the suspected phosphine. The cultures were 4 to 5 days old when their atmosphere was flushed out. A more sensitive chemical method reported for the determination of phosphine in air was then employed (13). The atmosphere from the Brewer jar containing 9 inoculated plates of the API strain was flushed with hydrogen through a trap containing glass beads and acidified (5% sulfuric acid) N/10 potassium permanganate solution to oxidize the phosphine to phosphate. After titration with N/10 oxalic acid to remove the excess permanganate, ammonium molybdate (2% solution) and stannous chloride solution (0.5%) were added to the acidified solution. An intense dark molybdenum blue color (indicating the presence of phosphate) was found to develop. Analysis of the blank solution (permanganate solution flushed with hydrogen) indicated the presence of only traces of phosphate.

In addition to flushing the gas through a trap containing an acidified permanganate solution, the solution in a petri dish was placed on top of a stack of inoculated plates in a Brewer jar. Analysis of this solution also revealed a much greater concentration of phosphate than a control solution in an atmosphere of hydrogen.

A volatile phosphorous compound was also detected from growth on plates of trypticase soy broth plus salts (Appendix) of one of the marine isolates of Desulfovibrio using acidified permanganate solution as the trapping agent.

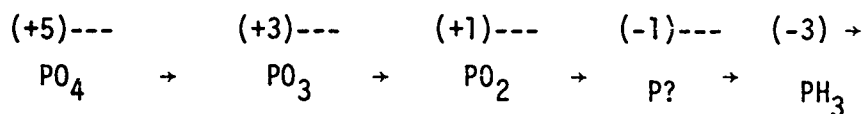
Phosphine may be detected by its reaction with mercuric chloride to form a yellow precipitate according to the following reaction:



A tan-colored precipitate (6.9 mg, dry wt.) was formed when a petri dish bottom containing 1%  $\text{HgCl}_2$  was placed on top of a stack of 9 inoculated petri plates containing Difco yeast extract (2%) agar (2%) and dipotassium phosphate (0.5 gm/200 m agar) after 11 days incubation. Comparison of this material with the precipitate formed by phosphine (generated by the action of an aqueous 50% sulfuric acid solution V/V on zinc phosphide) on  $\text{HgCl}_2$ , by X-ray diffraction has revealed them to be dissimilar. The X-ray pattern for the yellow product produced by the action of phosphine on  $\text{HgCl}_2$  appeared identical to that of  $\text{HgCl}_2$ . The pattern for the tan powder precipitated by the

bacterially produced gas in  $\text{HgCl}_2$  showed certain similarity to  $\text{Hg}_3\text{PO}_4$ . It is now strongly suspected, however, that the reactive produce, whereby iron phosphide may be formed, if indeed this is the mechanism, is not phosphine but another intermediate, volatile, phosphorous containing product formed in the reduction of phosphate. Phosphine does not blacken the agar media containing ferrous salts as does the gas produced by the organisms. If this blackening of the agar is due to iron phosphide (and this is being investigated), positive evidence of the mechanism of its formation would definitely be indicated. It was also of interest that no distinct odors of hydrogen sulfide or phosphine could be detected upon opening the culture jars.

The tan-colored compound appears also to be produced in  $\text{HgCl}_2$  by the API strain growing in Difco yeast extract (2%) agar containing hypophosphite ( $\text{NaH}_2\text{PO}_2$ ). This might indicate that the suspected intermediate is produced after the reduction of hypophosphite. Assuming changes of two electrons in each stage of reduction, since all the known hydrogen carried in the bacterial cell such as pyridine nucleotides can apparently accommodate only two hydrogen atoms or electrons at a time, the steps in the reduction of phosphate would be as follows



Postulated intermediates in which phosphorous would have a valence of -1 would include PH and  $H_3PO$ . The suggestive evidence from the X-ray pattern indicates that oxygen may be present and that the latter compound would be a greater possibility. Many of these intermediates, however, are very short lived. Phosphite ( $PO_3^{--}$ ) and hypophosphite ( $PO_2^{--}$ ) have been reported, however, as intermediates in the reduction of soil bacteria (12), using a paper chromatographic technique. The formation of these compounds by Desulfovibrio, marine and non-marine strains, will also be investigated.

Phosphine, as produced by the action of dilute acid on zinc phosphide was found to produce blackening of lead acetate paper, primarily around the edges. Blackening of lead acetate paper was also noted when dilute acid was added to the commercial sample of iron phosphide ( $Fe_2P$ ), probably due to other phosphides of iron or other metals as most of the iron phosphide did not react. This observation appears interesting since one of the tests used in the field for detecting iron sulfide (indicating bacterial corrosion) is to add a few drops of concentrated acid to black corrosion products and test the resulting gas for the presence of hydrogen sulfide using lead acetate paper. Usually the odor of hydrogen sulfide can also be detected. It has been noted by one of the investigators in soil corrosion at the National Bureau of Standards that occasionally

blackening of lead acetate paper occurs without any detectable odors of hydrogen sulfide. This is suggestive evidence that phosphides might be formed as corrosion products of bacterial action in the field. Further investigations in this area will be carried out as the opportunity arises.

(d) Other Possible Electron Acceptors in the Cathodic Depolarization of Iron

A preliminary study of nitrate and nitrite as electron acceptors (in addition to the dye benzyl viologen and phosphate), which might be utilized by Desulfovibrio in the cathodic depolarization of steel, was initiated. Nitrite has been reported by Senez et al as an excellent electron acceptor for Desulfovibrio (18). They did not, however, find nitrate to be reduced. Baumann and Denk did find nitrate reduction to be rapid and quantitative when lactate was the hydrogen (electron) donor (19). The technique previously described (2) was employed in which a steel coupon is placed on the surface of agar (containing an organic buffer plus an electron acceptor to be studied) with one portion resting on cells of the organism to be studied. Assuming no complicating secondary reactions and that a satisfactory electron acceptor is present, the organisms have been known to remove hydrogen or electrons from the surface of the steel and reduce the electron, benzyl viologen acceptors, at the cathode thus causing ferrous ions to go into solution at the anode (where they can be demonstrated



by adding ferricyanide to the surface of the agar).

Nitrite ( $\text{NaNO}_2$ ) and nitrate ( $\text{KNO}_3$ ) were employed as potential hydrogen acceptors in concentrations of 10 mg per 20 ml of washed agar (BBL) containing 0.01 M tris buffer adjusted to  $\text{pH } 7.0 \pm 0.1$ . Control plates with no electron acceptors were also prepared. Six day old cells of the API strain were used and the plates with the coupons placed in a Brewer jar with a helium atmosphere at  $28^\circ\text{C}$ . After 18 hours the plates and coupons were removed. Ferrous ions were detected in the agar at both cathodic areas (bacterial cells) and anodic areas with nitrate as a potential hydrogen acceptor. As there seemed to be no difference between the concentration of ferrous ions at both anodic and cathodic areas in the control plates (no electron acceptors), as evidenced by the amount of colored ferrous ferricyanide complex, it appeared that nitrate was not utilized as an electron acceptor. No ferrous ions were detected in the agar containing nitrite ions. This might have been expected as nitrite is a known corrosion inhibitor for iron. Since phosphate is a corrosion inhibitor for iron and it appears to be reduced in yeast extract in contact with iron, it is possible that yeast extract was interfering with the corrosion inhibition action of phosphate. Further investigation is contemplated in this area.

### 3. Effect of Microorganisms on the Corrosion Process in a Marine Environment

In a study of microbial corrosion in the marine environment, it

is theoretically desirable to compare the corrosion rate in the presence and in the absence of microorganisms, all other factors being equal. Practically, however, this may be difficult to accomplish.

As many structures in the marine environment are cathodically protected, it was first decided, however, to study the effects of microorganisms on such a system. Various circuits which might be used were first investigated. For these investigations a 3% sodium chloride solution in tap water was used instead of sea water. It soon became evident that light had a predominant effect on the protection current. A preliminary study of this factor was initiated.

(a) Effect of Light on Cathodic Protection of Steel in Salt Water

All of these investigations were carried out in succession on a single mild steel rod electrode (7.7 cm long and 1.27 cm in diameter) totally immersed in a 4 1/2 gallon jar (12" high and 12" in outer diameter) containing 17 liter of tap water plus sodium chloride (3%). The jar was placed on a table so that the electrode was 4'9" from a window facing the north. The auxiliary electrode consisted of a piece of completely immersed platinum foil (1 x 5 cm) attached to a platinum wire. The electrodes were suspended by a plexiglass cover which also prevented evaporation. Holes in the cover permitted introduction of a thermometer and a commercially available calomel half cell. The first circuit employed is shown in Figure 3. The resistance of 1500 ohms

was selected to maintain an initial potential on the steel electrode of 0.8 volts. Variations in the potential and current were soon detected and it appeared that these variations were related to the light intensity. During the night and early in the morning the potential remained about 0.84 volts and the current about 0.48 mA. When the room was fully illuminated during the day the potential dropped to about 0.74 volts and the current increased to about 0.60 mA. No relation to the temperature could be detected.

A second circuit was then employed (Figure 4). The changes in current with illumination were even more pronounced as indicated in Figure 5. During the daylight the changes in current appeared to be too sensitive to changes in light intensity caused by the passage of clouds. In several experiments the venetian blind was closed and the changes in current measured after they were opened. This effect is shown in Figure 6. The effect of short bursts (2 min.) of high intensity artificial light (commercially available halogen bulb used for photography) on the current is indicated in Figure 7. The light source was placed about 3 feet from the electrode. Again this effect could not be related to temperature changes.

In order to maintain a fixed potential on the electrode, the circuit (potentiostatic) indicated in Figure 8 was then employed. The potential on the electrode was set at 0.8V by means of the variable 1000 ohm resistance and was maintained to within  $\pm 0.02V$  during the

experiments. Over a consecutive period of seven days the current required to maintain the potential at 0.8V generally remained at 0.29 mA ( $0.90 \text{ mA/dm}^2$ ) and rose during the day to a maximum of about 0.44 mA ( $1.37 \text{ mA/dm}^2$ ) during the period of greatest light intensity in the day. On the basis of these results about 52% more current or 1.5 times the amount of current required in the dark was needed to maintain the same potential (0.8V) in the light.

(b) Effect of Light on Corrosion of Steel in Salt Water

The influence of light upon the corrosion rate was then determined by a polarization technique using the circuit in Figure 9. Three polarization curves (anodic and cathodic) were obtained from data recorded on three days (not successive) with the room lights off and the blind closed. Three other curves were obtained on bright days with the blind open and the room lights off. The data for the cathodic curves were obtained in the morning and the data for the cathodic curves in the afternoon with an interval of at least two hours between. The current was applied in increments of 0.5 mA for a duration of two minutes. The potential was recorded at the end of this duration.

Corrosion rates were calculated from the polarization curves plotted on coordinate paper by the so-called "polarization break" method (20). The "break" in the cathodic curve is the point  $I_c$ , the intersection of tangents to the two slopes in the curve. The point  $I_a$  is the intersection of the tangents to the two slopes in the anodic

curve. The corrosion current is calculated from the Pearson equation (21):

$$i_o = \frac{I_c I_a}{I_c + I_a}$$

The corrosion currents and rates for the three dark periods and the three light periods are presented in Table 1. The data for a bright and a dark period are presented in Figure 10. Using the average values, the corrosion rate in the light is about 1.3 times the corrosion rate in the dark or about 28% greater in the light than in the dark. The corrosion ratio is about 100 to 77. Corrosion ratios of 100 to 83.3 and 84.6 have been reported for light and darkness based upon weight losses (22). The temperature varied from 22.5 to 23°C during these measurements and there did not appear to be any correlation between temperatures and corrosion rate.

The potential of the corroding steel was usually found to be displaced 10-15 mV in a more positive direction during the light period. This is also in agreement with some earlier results (22).

In addition to the effect of light on the corrosion process, the velocity of the water appears to be another factor to be considered in such an investigation (23). Future investigations will attempt to investigate the effect of stagnant sea water (harbor water); however, cathodic protection and the corrosion rate of steel as attacked by microorganisms may be more severe in this environment.

### SUMMARY

1. The inability to grow non-marine sulfate reducers (Desulfovibrio) successfully on the agar surface of media (Baar's and yeast extract) used for their cultivation has been found to be due to the inhibitory effect of the indicator salt (ferrous ammonium sulfate) for hydrogen sulfide in the media.
2. Trypticase soy agar plus salts medium (used successfully for the isolation of non-marine Desulfovibrio strains) was not found to be suitable for the isolation of pure cultures of marine strains (Desulfovibrio). Development of a suitable medium for direct counting and isolation of marine strains of Desulfovibrio will be continued.
3. An agar (2%) medium consisting only of yeast extract (2%) was found to be useful as a screening agent for hydrogen (electron) donors and acceptors for the growth of Desulfovibrio.
4. Phosphate was found to be a suitable electron acceptor for growth in yeast extract agar in the presence of hydrogen as an electron donor.
5. A volatile phosphorous product was detected during the growth of Desulfovibrio on yeast extract agar containing phosphate. The compound is not phosphine, but appears to contain oxygen. Hydrogen may also be present. It appears to be a product of phosphate reduction and may be involved in the reaction with ferrous ions to produce iron phosphide.

Preliminary evidence indicates that marine strains of Desulfovibrio may also produce this volatile material. The exact composition of this compound will be determined.

6. The black colloidal material produced in yeast extract cultures of Desulfovibrio containing mild steel was found to be iron phosphide.

7. Crystals of vivianite ( $\text{Fe}_3(\text{PO}_4)_2 \cdot 8\text{H}_2\text{O}$ ) were found to develop on mild steel specimens in inoculated (Desulfovibrio) yeast extract broth after 1 month incubation in a hydrogen atmosphere.

8. In preliminary studies to determine the effect of microorganisms on cathodically protected steel it was found that light has an effect upon the current required for protection. On the steel specimen employed, it was found that 1.5 times the amount of current required in the dark was needed to maintain the same potential (0.8V) in the light.

9. The corrosion rate in indirect sunlight was found to be about 1.3 times the corrosion rate in the dark.

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The X-ray diffraction studies by C. Bechtoldt and the Mössbauer studies by Dr. J. Spijkerman are most gratefully appreciated.

### Publications and Talks Resulting from Research

Part of the work will be included in a paper to be presented at the 1st International Biodeterioration Symposium to be held in Southampton University, England, in September 1968.



## REFERENCES

1. C.A.H. von Wolzogen Kühr and L.S. van der Vlugt, Water 18, 147 (1934).
2. W.P. Iverson, Science 151, 986 (1966).
3. R.L. Starkey and J.D. Schenone, J. Bacteriol. 54, 46 (1947).
4. R.L. Starkey, Bacteriol. Proc. 51 (1948).
5. R.L. Starkey, Intern. Congr. Microbiol. Rept. Proc., 6th Congr. 3, 326 (1953).
6. E.E. Corcoran and J.S. Kittredge, Marine Corros. & Fouling Conf., Scripps Inst. of Oceanography, La Jolla, Calif., April 18-20, 1956.
7. W.G. Blaston and C.H. Oppenheimer, Bacteriol. Proc. 36 (1960).
8. W.P. Iverson, Science 156, 1112 (1967).
9. A.M. Saleh, R. Macpherson and J.D.A. Miller, J. Appl. Microbiol. 27, 281 (1964).
10. W.P. Iverson, Nature 217, 1265 (1968).
11. K.I. Rudakov, Zentr. Bakteriolog. Parasitenk. Abt. II, 79, 229 (1929).
12. G. Tsubota, Soil Plant Food (Tokyo) 5, 10 (1959).
13. J. Borrowdale and C.E.A. Shanahan, Ann. Occup. Hyg. 4, 36 (1961).
14. L. Moser and A. Brukl, Z. anorg. allgem. chem. 121, 73 (1922).
15. P. Lemhoult, Compt. rend., 145, 1175 (1907).
16. M. Wilmet, Compt. rend. 185, 206 (1927).
17. K. Beyer, Z. anorg. allgem. chem. 250, 312 (1943).

18. J.C. Senez, F. Pichinoty and M. Konavoltchikoff-Mozoger, Compt. rend., 242, 570 (1956).
19. A. Baumann and V. Denk. Arch. Mikrobiol. 15, 283 (1951).
20. W.J. Schwerdtfeger, J. Research, NBS 65, Oct.-Dec. (1961).
21. J.W. Pearson, Trans. Electrochem. Soc. 81, 485 (1942).
22. C.O. Bannister and R. Rigby, J. Iron & Steel Inst. 133, 293 (1936).
23. C.A. Smith, Meeting, National Association of Corrosion Engineers, March 18-21 (1968).

## APPENDIX

### Pankhurst's medium\*

$K_2HPO_4$	0.5 gm.
$NH_4Cl$	1.0 "
$CaSO_4$	1.0 "
$MgSO_4 \cdot 7H_2O$	2.0 "
Sodium lactate	5.0 "
Tap water	930 ml

pH adjusted to 8.1 before sterilizing.

Sterilized by autoclaving 10 lbs/sq in. for 20 min.

plus 50 ml of a 1% (w/v) solution of  $FeSO_4(NH_4)_2SO_4 \cdot 6H_2O$

(sterilized by steaming 1 hr. on 3 successive days,

plus 10 ml of a 10% (w/v) solution of Difco yeast extract

(sterilized by autoclaving)

plus 10 ml of a 1% (w/v) solution of sodium thioglycollate

(sterilized by membrane filtration)

pH of medium after prep. = 7.2 - 7.6

\*Baars, J.K. (1930) Over Sulfaatreductie door Bacteriën Dissertation.

Delft: W.D. Meinema

Trypticase Soy Agar Plus Salts Medium\*

+Trypticase Soy Agar (BBL)	40.0 gm
Sodium lactate (6% sol.)	4.0 ml
Magnesium sulfate	2.0 gm
Agar	5.0 "
Sodium Chloride	25.0 "
Ferrous ammonium sulfate	0.5 "
Distilled water	1000.0 ml

Ferrous ammonium sulfate solution (10 ml dist. H<sub>2</sub>O) sterilized  
by Seitz filtration. pH adjusted to 7.0 ± 1.

+Trypticase Soy Agar (BBL)\*

Trypticase	15.0 gm
Phytone	5.0 "
Sodium Chloride	5.0 "
Agar	15.0 "
Distilled water	1 liter

\*W.P. Iverson, J. Appl. Microbiol. 14 (4) 529-534 (1965).

API Medium\*

Sodium lactate (60%)	4.0 ml
Yeast extract	1.0 gm
Ascorbic Acid	0.1 "
MgSO <sub>4</sub> ·7H <sub>2</sub> O	0.2 "
K <sub>2</sub> HPO <sub>4</sub> (Anhyd)	0.01 gm
Fe(SO <sub>4</sub> ) <sub>2</sub> (NH) <sub>2</sub> ·6H <sub>2</sub> O	0.1 "
NaCl	10.0 "
Distilled water	1000 ml

pH adjusted to 7.2 - 7.5 with NaOH

\*R.C. Allred, Prod. Month. 22, 32 (1958)

TABLE 1

The Effect of Light on the Corrosion Rate of Steel

<u>Day</u>	<u>Dark</u>		<u>Light</u>	
	<u>Corrosion Current (<math>i_o</math>) (mA)</u>	<u>Corrosion Rate<sup>+</sup> (mA/dm<sup>2</sup>)</u>	<u>Corrosion Current (<math>i_o</math>) (mA)</u>	<u>Corrosion Rate (mA/dm<sup>2</sup>)</u>
22 Jan	---	---	0.34	1.06
23 Jan	0.24	0.75	---	---
25 Jan	0.23*	0.72	---	---
26 Jan	---	---	0.26	0.81
5 Feb	---	---	0.27*	0.84
6 Feb	<u>0.20</u>	<u>0.62</u>	<u>---</u>	<u>---</u>
Average	0.22	0.70	0.29	0.90

+Area of electrode = 32 cm<sup>2</sup>

$$\frac{i_o}{32\text{cm}^2} \times 100\text{cm}^2 = \text{mA/dm}^2$$

\*Data plotted in Figure 10

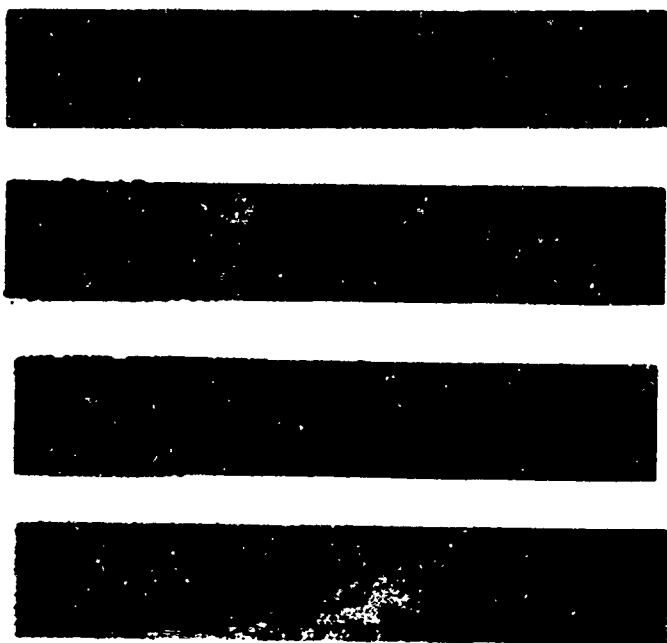


Fig. 1. Crystals of vivianite on 1010 steel coupons X 1.25. Coupons were in a vertical position while in the yeast extract broth (right → left = top → bottom).



Fig. 2. Crystals of vivianite on 1010 steel coupon. X10

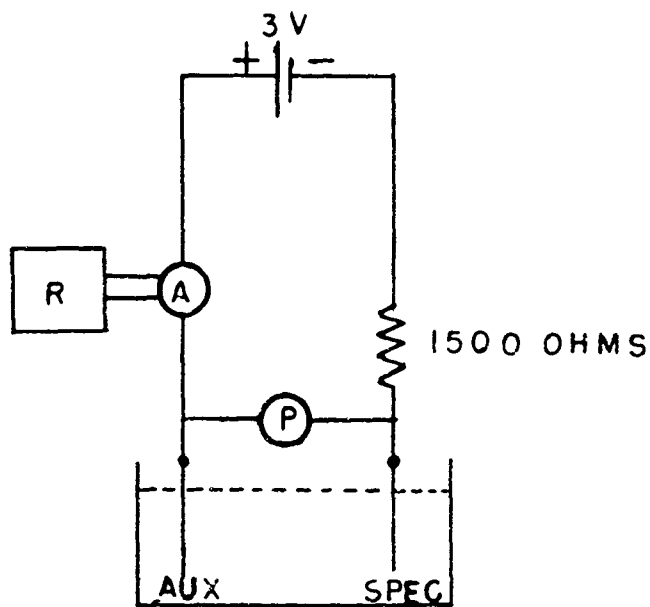


Fig. 3. Diagram of 1st circuit used for cathodic protection.  
 R = recorder, A = ammeter, P = electrometric potentiometer,  
 AUX = platinum auxiliary electrode, SPEC = steel rod.



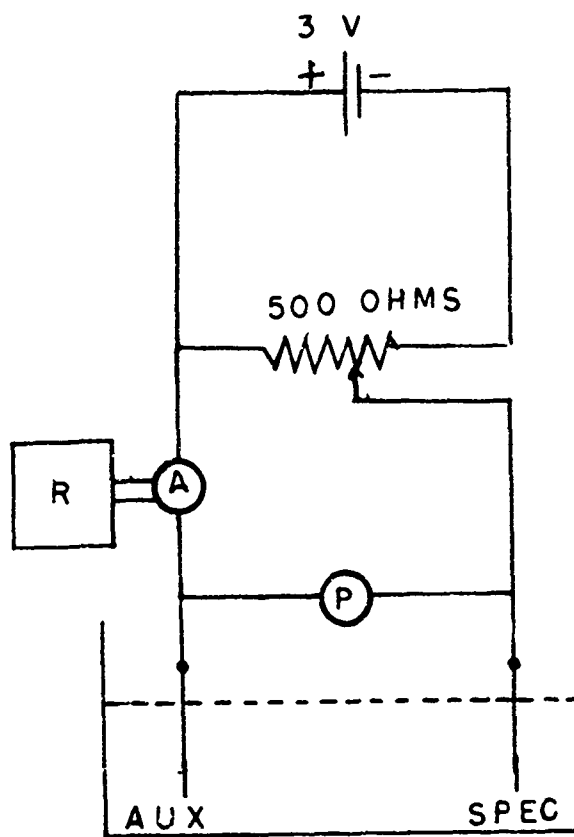


Fig. 4. Diagram of 2nd circuit used for cathodic protection.  
 R = recorder, A = ammeter, P = electrometric potentiometer,  
 AUX = auxiliary platinum electrode, SPEC = steel rod.

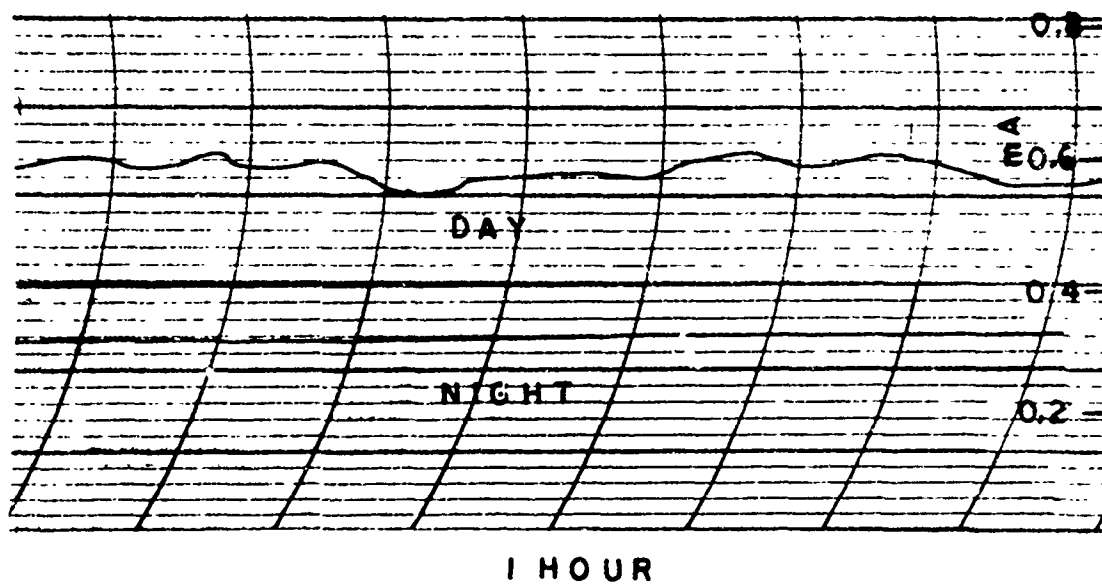


Fig. 5. Recorder tracings of cathodic protection current at night and during the day (during a 1 hour interval).

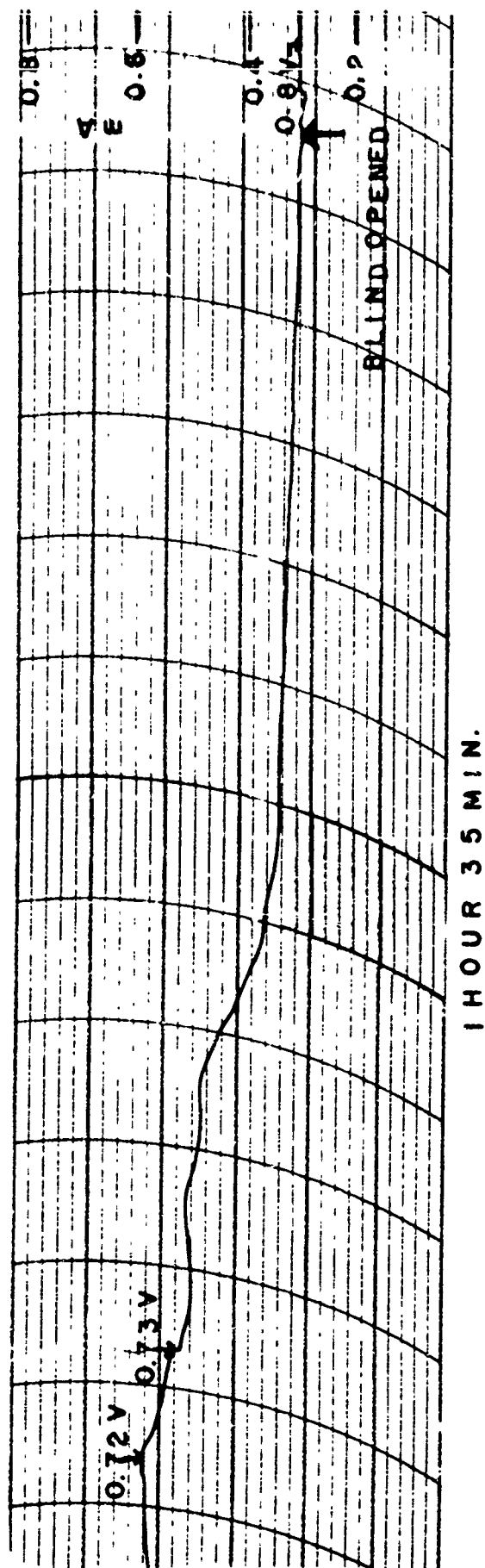


Fig. 6. Recorder tracing of cathodic protection current after admittance of sun light to the electrode, during 30 interval of 1 hour, 35 minutes. Blind closed for a period of 17 hours prior to opening. Potential indicated by arrows.

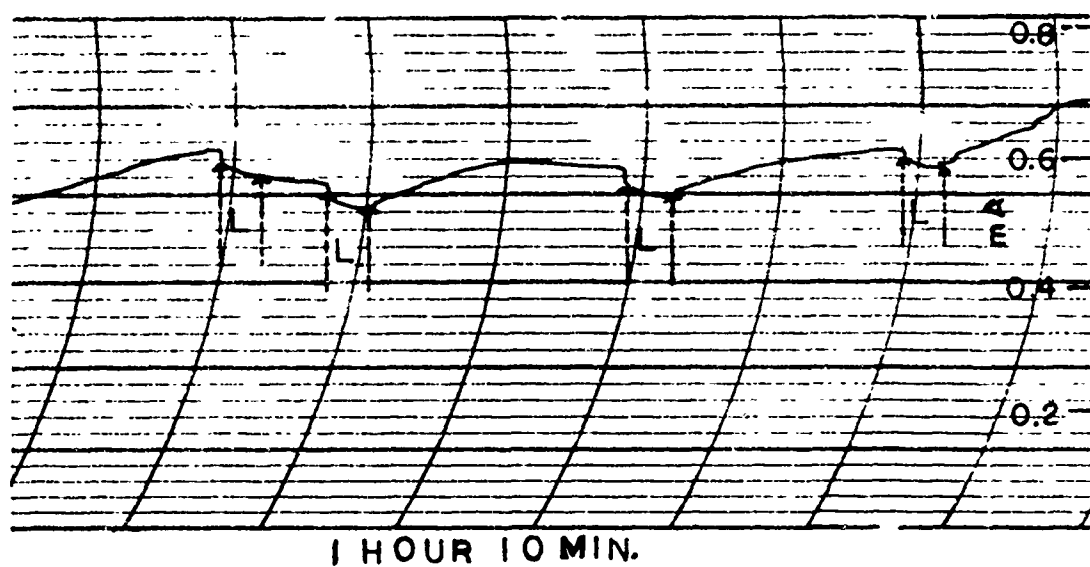


Fig. 7. Effect of high intensity light from halogen lamp on cathodic protection current. Potential remained at 0.74V. L = 2 minute period of light exposure.

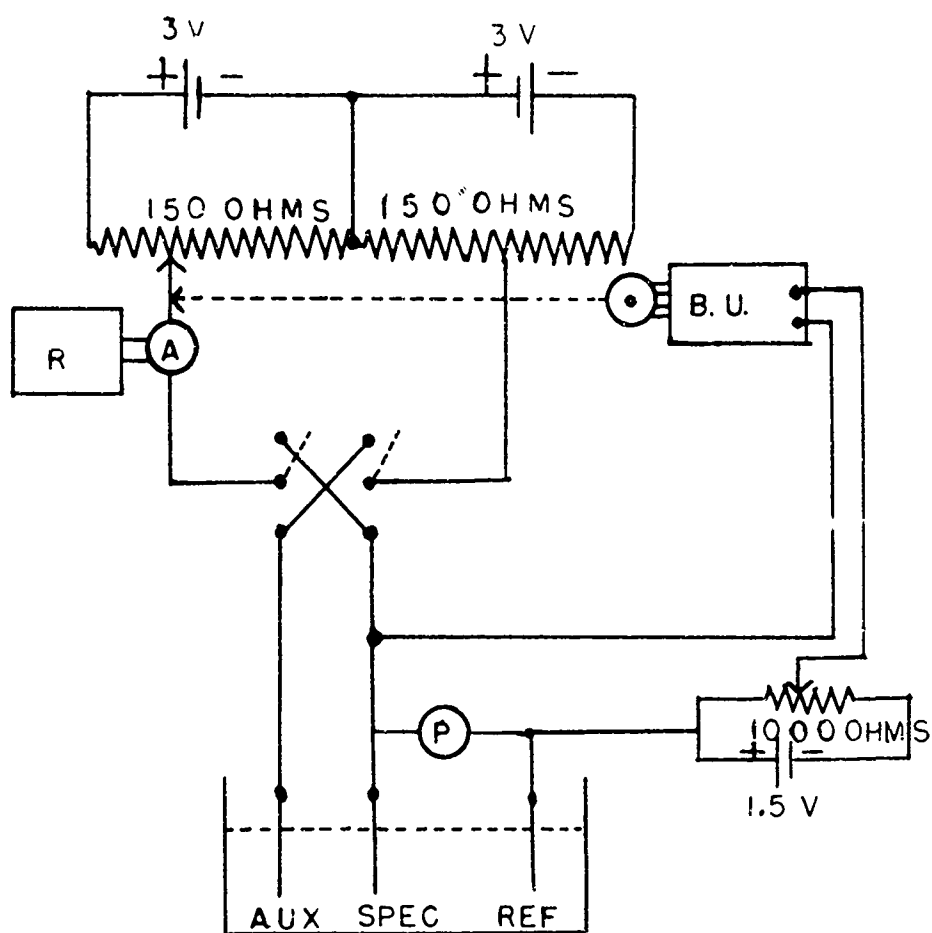


Fig. 8. Diagram of potentiostatic circuit for cathodic protection. R = recorder, A = ammeter, P = electrometric potentiometer, B.U. = balancing unit, AUX = auxiliary platinum electrode, SPEC = steel electrode, REF = calomel reference electrode.

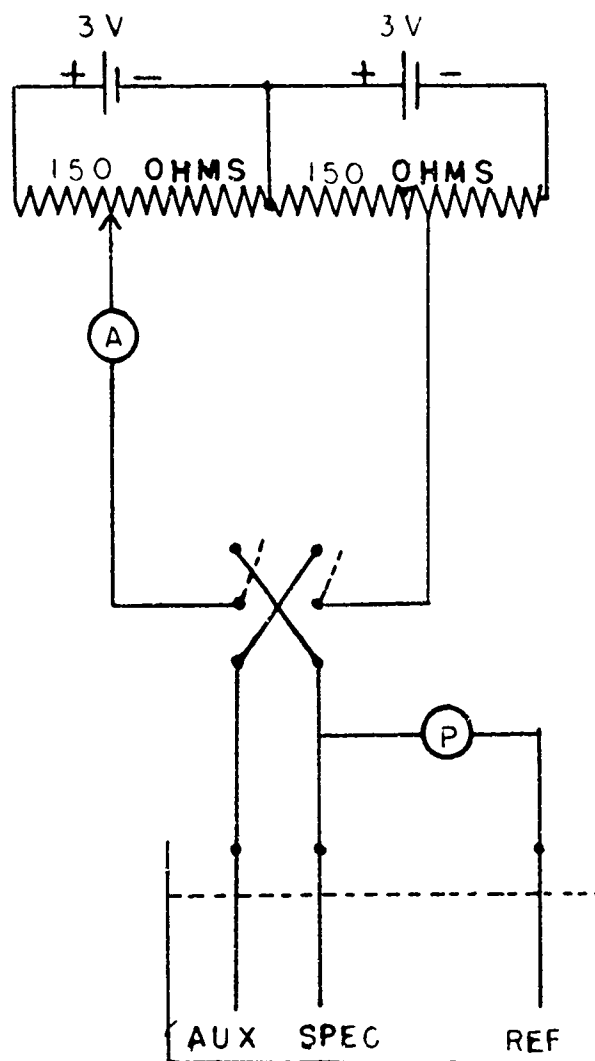


Fig. 9. Diagram of polarization circuit. A = ammeter, P = electrometric potentiometer, AUX = platinum auxiliary electrode, REF = calomel reference electrode, SPEC = steel rod specimen.

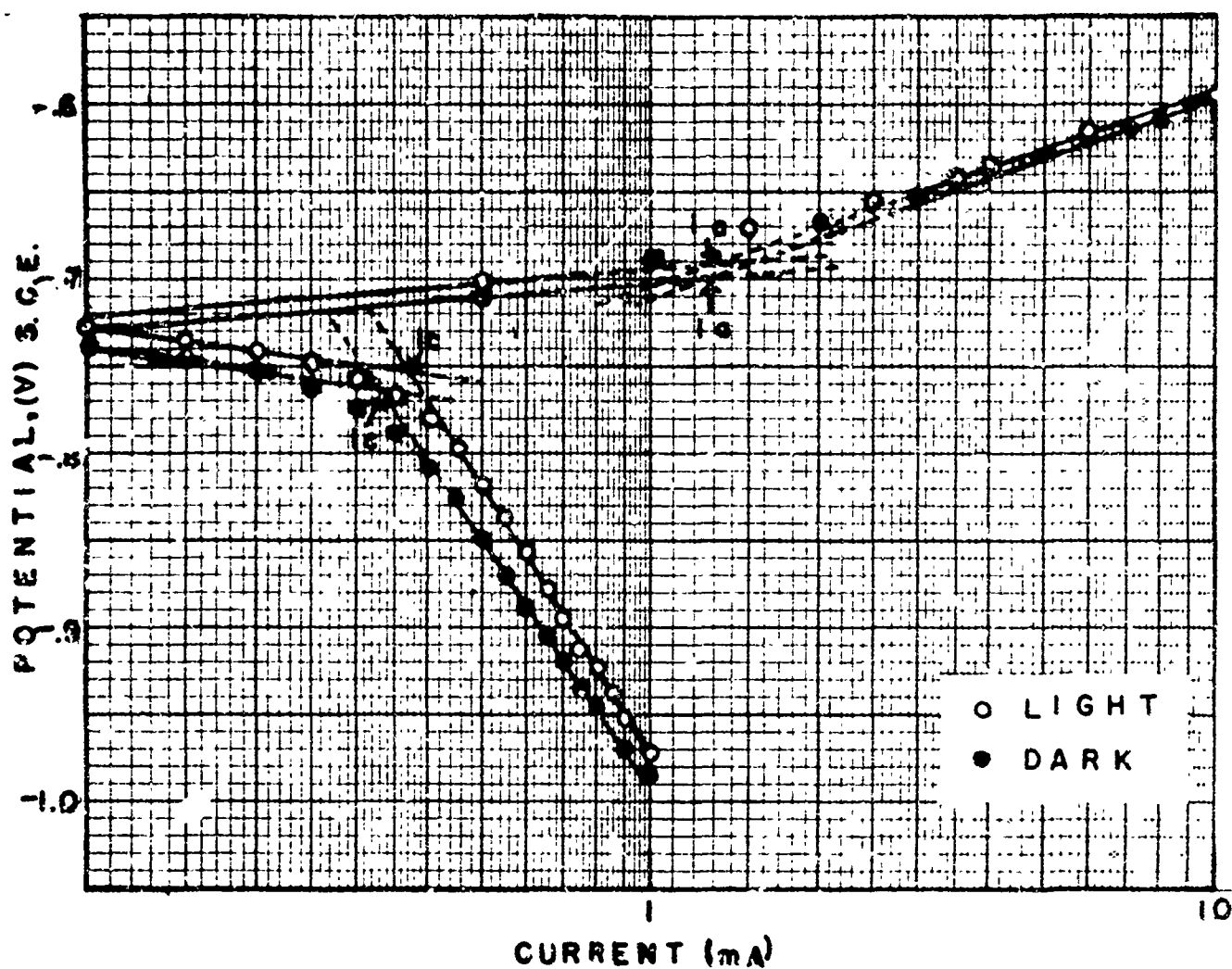


Fig. 10. Effect of light on polarization curves. Several values for anodic curves not indicated.

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13. ABSTRACT Studies of the effect of microorganisms, particularly sulfate reducers ( <u>Desulfovibrio</u> ), on marine corrosion were initiated. The inability of sulfate reducers to grow on the agar surface of media appears to be due to the presence of the ferrous salt used as an indicator for hydrogen sulfide. Evidence indicates that phosphate may be an electron acceptor in the cathodic depolarization of steel. Iron phosphide ( $\text{Fe}_3\text{P}$ ) and vivianite ( $\text{Fe}_3(\text{PO}_4)_2 \cdot 8\text{H}_2\text{O}$ ) were produced by <u>Desulfovibrio</u> growing in contact with mild steel. The organism reduces phosphate in the presence of hydrogen to form a volatile phosphorous containing compound which is not phosphine. Light increases the corrosion rate of steel as indicated by polarization measurements. The cathodic protection currents required to maintain a potential of 0.8 volts on a steel specimen in indirect sunlight was found to be 1.5 times that required in the dark.		

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